ATTACHMENT C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	C.L. Steele et al.	Attorney Docket No.:	WSUR118414
Application No.:	10/025,145	Group Art Unit:	1638
Filed:	December 19, 2001	Examiner:	C.E. Collins
Title:	MONOTERPENE SYNTHASE FROM GRAND FIR (ABIES GRANDIS)		

DECLARATION OF RODNEY B. CROTEAU

TO THE COMMISSIONER FOR PATENTS:

RODNEY B. CROTEAU hereby declares that:

1. I am the Rodney B. Croteau named as an inventor of the above-captioned patent application, and I am familiar with the subject matter disclosed and claimed therein.

2. It is my understanding that the Examiner argues that the effect of expressing a nucleic acid encoding a (-)-camphene synthase in any unspecified eukaryotic cell is unpredictable, since monoterpenes such as camphene, that would be produced as a consequence of (-)-camphene synthase expression, are known to be toxic to certain types of eukaryotic cells.

3. As described herein, my colleagues and I successfully expressed a (-)-camphene synthase cDNA, and produced (-)-camphene, in yeast.

4. The (-)-camphene synthase cDNA clone identified as SEQ ID NO:64 in the above-referenced patent application, was transferred, by standard polymerase chain reaction and restriction/ligation methods, from plasmid pSBETa to the yeast expression vector pYES-NT under control of the GAL10 promoter.

5. The resulting pYES-NT construct (containing the (-)-camphene synthase cDNA) was introduced into *Saccharomyces cerevisiae* strain YPH499. The recombinant yeast (containing the pYES-NT construct) were grown in 100 ml culture medium, at 31°C, to an optical density of about 0.4, and then expression of the (-)-camphene synthase cDNA was induced with galactose.

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6. The recombinant YPH499 cells were grown in the presence of galactose overnight. The cells were then harvested by centrifugation, homogenized in assay buffer containing divalent metal ions, and the soluble enzyme fraction was prepared by centrifugation.

7. An assay was performed to determine whether the soluble enzyme fraction, isolated from the recombinant YPH499 cells, included (-)-camphene synthase. An aliquot of the soluble enzyme fraction was incubated, for two hours at 30°C, in the presence of the substrate geranyl diphosphate, and the resulting terpenoids were extracted, purified by column chromatography, and analyzed by gas chromatography and mass spectrometry (GC-MS).

8. The recombinant YPH499 cells produced (-)-camphene synthase at a concentration of approximately 3 mg/liter culture medium (based on specific activity). The amount of (-)-camphene produced by the recombinant YPH499 cells was approximately 50 micrograms camphene/liter culture medium. A soluble enzyme fraction from control YPH499 cells (that did not include a nucleic acid molecule encoding a (-)-camphene synthase), did not produce detectable (-)-camphene.

9. I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: 5 JAN 05

Rodney B. Croteau

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